Rejection Under 35 U.S.C. § 112, First Paragraph

In the Advisory Action dated October 11, 2001 (Paper No. 12), the Office maintained the rejection of claims 40-58 under 35 U.S.C. § 112, first paragraph, alleging that applicants have not disclosed a particular phenotype resulting from expression of a transgene in a mouse. (Paper No. 12, page 2.) The Office's enablement attack of applicants' claimed invention is two-fold. First, in view of the references cited and arguments set forth in the Office Actions dated June 6, 2001, and September 28, 2000, the Office takes the position that the phenotype resulting from the expression of a transgene is unpredictable and "dependent on the particular nucleotide sequence, operably linked to a specific promoter, encompassed within the transgene as well as the site of integration of the transgene." (*Id.*) Second, the Office asserts that one of skill in the art would not know how to use a transgenic mouse that allegedly lacks a corresponding phenotype. (*Id.*) The Office acknowledges that one of skill in the art would be able to make a transgenic mouse expressing a transgene of interest, as claimed. Thus, "[t]he issue is not the ability to create a transgenic mouse but rather whether the specification has taught a use for such a transgenic mouse in the absence of a recited phenotype." (*Id.*) Applicants respectfully traverse this rejection.

1. **Predictability of the Claimed Invention**

The Office contends that the phenotype resulting from the expression of a transgene in a mouse, within the scope of applicants' claims, is unpredictable. (Paper

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No. 12, page 2.) This contention is premised on the Office's assertions that the phenotype of a transgenic mouse generally is (1) "dependent on the particular nucleotide sequence, operably linked to a specific promoter, encompassed within the transgene" that is placed into the transgenic mouse's genome and (2) dependent on "the site of integration of the transgene" in the mouse's genome. (*Id.*) Even assuming, *arguendo*, that both of these points are scientifically true, and applicable to transgenic mice in the abstract, applicants' respectfully submit that neither point is relevant to the inquiry as to whether applicants' claims, to particular transgenic mice, are enabled.

The phenotype of any transgenic mouse, as it deviates from wild type, is determined by the transgenic construct that it comprises. A transgenic construct comprises at least (1) a promoter, that is operatively linked to (2) a nucleotide sequence encoding a polypeptide. The nucleotide sequence determines the identity of the encoded polypeptide, which in turn, based on its function or activity, imparts a novel phenotype to all or a subset of the cells of the transgenic mouse. This latter point, namely, which cells of the mouse will express the polypeptide and, therefore, express a novel phenotype, is determined by the identity of the promoter sequences, which are operatively linked to the nucleotide sequence, which encodes the polypeptide.

Applicants' claims define both the promoter and the nucleotide sequence encoding a polypeptide, which are present in the DNA sequences used for each of the processes (i.e., claims 46, 47, and 51-58), and which are present in each of the transgenic mice (i.e., claims 40-45 and 59-62), of the invention. Applicants' disclosure,

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coupled with the prior art, makes clear that all of the embodiments encompassed by the claims are enabled. Therefore, it is irrelevant whether some *unclaimed* transgenic constructs might exist that are not enabled.

In all of the claims, the promoter sequences used are "the sequence from about nucleotide -1125 to about nucleotide +38 as set forth in Figure 1 (SEQ ID NO. 22)." (*E.g.*, claim 40.) The nucleotide sequence encoding a polypeptide of the claims is one that encodes a heterologous polypeptide that can be one or more of an oncogenic, tumorigenic, or immortalizing protein; a reporter gene; or luciferase or β-galactosidase, depending on the claim.

Applicants' disclosure describes the expression pattern of a β-galactosidase reporter gene linked to a promoter, which falls within the scope of a promoter sequence that is "the sequence from about nucleotide -1125 to about nucleotide +38 as set forth in Figure 1 (SEQ ID NO. 22)," as required by the claims. Specifically, the specification describes that, "the 1163 bp promoter [was tested] in vivo" by linking it to "the nls-β-galactosidase reporter gene" and microinject[ing it] into the male pronuclei of fertilized eggs from F1 hybrid mice" to make transgenic mice comprising the transgene. (Specification, p. 38, lines 17-19.) The results presented in the specification show that two of three transgenic founders expressed the product of the β-galactosidase reporter gene. These results further demonstrate that, in the two expressing lines, expression in the peripheral nervous system was the same, while, in contrast, expression in the central nervous system of one of the lines (line 26) was a subset of that of a second line

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(line 13). (*Id.* at page 38, line 25 – page 39, line 7.) The description of the expression pattern of this transgene that is presented in the specification focuses on transgenic line 13, and it was noted that, "[a]s expected, most peripheral β2-expressing ganglia expressed β-galactosidase (β-gal), whereas in the CNS only a subset of β2-positive regions expressed the β-gal." (*Id.* at page 39, lines 7-10.) Thus, applicants' newly discovered promoter confers the expression pattern of the β2-subunit of the neuronal nicotinic acetylcholine receptor to the β-galactosidase coding sequences in the transgene.

This result shows unequivocally that applicants' promoter has the ability to drive the expression of a "nucleotide sequence encoding a heterologous polypeptide . . . in neurons of the transgenic mouse," as required by the claims. Thus, these elements of the claims are clearly enabled.

It was well known in the art that once an endogenous promoter was identified that could regulate the expression of a reporter gene in transgenic mice in a pattern that was characteristic of the expression pattern of the endogenous gene from which the promoter was derived, that promoter could be linked to a nucleotide sequence encoding a heterologous polypeptide, in order to cause the heterologous polypeptide to be expressed in the cells or tissues of the mouse in which the endogenous gene from which the promoter was derived was normally expressed. (*See, e.g.,* Aguzzi et al.[1] and

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[1] Aguzzi et al. has been discussed in further detail in applicants' previously filed Responses dated March 28, 2001, and August 30, 2001.

Camper et al.[2]) Nonetheless, the Office relies on a number of references that identify specific aspects of transgene constructs, such as the inclusion of introns, which are capable of increasing transgene expression in certain instances, as evidence that undue experimentation is required to make transgenic mice (*See, e.g.,* Palmiter et al., Kappel et al., and Cameron). Applicants respectfully submit that these references, while relevant to the general issue of how different aspects of transgene construction can impact the ability of a given transgene to be expressed, are not relevant to the issue of whether applicants' claimed transgenes can be expressed in transgenic mice. Specifically, applicants' disclosure already shows that their promoter confers the normal expression pattern of the β2-subunit of the neuronal nicotinic acetylcholine receptor to the β-galactosidase coding sequences in the transgene. Thus, this promoter is sufficient to express a heterologous polypeptide in neurons of the transgenic mouse, as required by the claims; no further experimentation is required.

The Office appears to consider the fact that not all mice that are transgenic for a particular construct will express the transgene encoded by the construct as evidence that an undue amount of experimentation is required to use transgenic mice. (Paper No. 12 at page 2.) Applicants submit that one of skill in the art was well aware that not all transgenic mice will express a transgene of interest. The skilled artisan, however, was willing and able to generate a number of transgenic mice and then screen them to find those that express the transgene—without undue experimentation. For example,

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[2] Camper et al. has been discussed in further detail in applicants' previously filed Responses dated March 28, 2001, and August 30, 2001.

Palmiter and Brinster[3], in a review on how to make and use transgenic mice, note that "the level of expression of a particular gene varies widely from one founder animal to another [ ], suggesting that chromosomal position can influence accessibility of the genes to transcription factors," and that "usually a few transgenic mice produced with any construct do not express the gene at all, which may be due to its integration into heterochromatin domains." Palmiter and Brinster, p. 473. It is also noted that, "the level of expression in some mice approaches or exceeds that of the endogenous genes, which indicates that optimal expression probably does not depend upon the normal chromosomal position." (*Id.*)

Applicants submit that one of skill in the art, in possession of applicants' disclosure that the promoter of the β2-subunit of the neuronal nicotinic acetylcholine receptor confers neuron-specific expression on heterologous coding sequences, would be prepared to generate multiple transgenic mice and then screen them to determine which ones express the transgene, and at which levels, in order to practice the claimed invention. *See, In re Wands*, 858 F.2d 7331, 740, 8 U.S.P.Q.2d 1400, 1406 (Fed. Cir. 1988) (finding that practitioners are prepared to screen negative hybridomas in order to find one that makes a desired antibody).

In summary, the specification demonstrates that applicants' promoter has the ability to drive the expression of a "nucleotide sequence encoding a heterologous polypeptide . . . in neurons of the transgenic mouse," as required by the claims. Since

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3 Palmiter and Brinster, Germ-Line Transformation of Mice, *Ann. Rev. Genet.*, Vol. 20, pp 465-99 (1986) (Exhibit A).

applicants' promoter regulates expression of a reporter gene in a pattern that is characteristic of the normal expression pattern of the endogenous β2-subunit of the neuronal nicotinic acetylcholine receptor, one of skill in the art would expect that applicants' promoter could be used to regulate expression of other heterologous sequences in a similar pattern. And although the site of transgene integration can affect the expression of the transgene, a skilled artisan could routinely screen the transgenic animals to determine those that express the transgene at what levels. Such routine screening does not amount to undue experimentation. Thus, the phenotype resulting from the expression of the DNA sequence in applicants' transgenic mice, as claimed, is not unpredictable.

2. The Specification Enables One of Skill in the Art How to Use the Claimed Invention

If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. § 112 is satisfied. M.P.E.P. § 2164.01(c). And when a compound or composition claim does not recite a specific use, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use. M.P.E.P. § 2164.01(c).

One aspect of applicants' invention involves promoter sequences of the β2-subunit of neuronal nicotinic acetylcholine receptor. Applicants have demonstrated that these promoter sequences can direct expression of a heterologous nucleotide sequence in a tissue-specific manner, both *in vitro*, and *in vivo* (for example, in

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transgenic animals). The promoters can direct expression of any heterologous sequence, or transgene, of interest. In one embodiment, the heterologous sequence encodes an oncogenic, tumorigenic, or immortalizing protein. (Specification, page 6, lines 6-14.) Using these newly discovered promoter sequences in combination with sequences encoding oncogenic, tumorigenic, or immortalizing proteins, applicants have created a novel transgenic system for directing neuron-specific tumor formation. As recited in the claims, the oncogenic, tumorigenic, or immortalizing protein is expressed in neurons of the transgenic mouse.

Kioussis[4] discusses the development of transgenic mice expressing oncogenes under the control of transcriptional elements, including tissue-specific promoters. As explained by Kioussis:

> These constructs are introduced in the germ line of mice, and animals that carry these genes usually express the transoncogene in the tissue determined by the regulatory elements of the hybrid gene. This tissue often suffers a developmental disturbance and *in most cases* a tumour develops from the cells that express the oncogene.

Kioussis, p. 196 (emphasis added). Kioussis then lists numerous examples of transgenic animals bearing an oncogene, including the SV40 large T antigen gene, the *myc* gene, a *ras*-activated gene, *neu*, and *fos*, under the control of a tissue-specific promoter. *Id.* Kioussis also notes that not all cells expressing the oncogenic transgene proceed to malignancy, suggesting the involvement of additional events, such as the

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4 Kioussis, *Oncogenisis and Transgenic Mice*, In: Grosveld, F. and Kollias, G. (eds.), Transgenic Animals, San Diego, CA, Academic Press, 1992: 195-210 (Exhibit B).

expression of "additional (onco) genes." *Id.* A transgenic animal, such as the mouse, however, provides the appropriate environment for these additional events. Indeed, referring to these transgenic mouse examples, Kioussis observes that "the animals expressing a trans-oncogene in their tissues almost *invariably* develop tumours." *Id.* at 204 (emphasis added).

Thus, the art recognized that tissue-specific expression of oncogenes in transgenic animals, including mice, would "invariably," or at least "in most cases," lead to tumor development, a recognizable phenotype. In the absence of evidence to the contrary, one of skill in the art would similarly expect that applicants' claimed transgenic mice, expressing an oncogenic, tumorigenic, or immortalizing protein in neurons, would "invariably," or at least "in most cases," develop tumors.

Gordon[5] also shows numerous examples of transgenic mice where sequences encoding oncogenic, tumorigenic, or immortalizing proteins were linked to various promoter and/or enhancer elements and expressed in specific tissues, causing tumor formation in the transgenic mice. Similarly, the phenotype of the claimed transgenic mice would include tissue-specific tumorigenesis in neurons. This transgenic system provides a useful animal model for testing anticancer drugs. (Specification, page 6.) Indeed, it was well recognized in the art that such transgenic animals provided a useful animal model for neoplastic disease, or cancer. According to Gordon:

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5 Gordon, *Transgenic Animals*, In: Bourne, GH, Jeon KW, Friedlander M (eds.), International Review of Cytology, San Diego, CA, Academic Press, 1989: 171-229 (Exhibit C).

> The ability to insert potentially oncogenic coding sequences into the mouse has allowed a novel approach to investigation of neoplasia. Linkage of such genes to appropriate promoter-enhancer elements can induce high levels of expression in specific tissues, which in turn can cause "directed tumorigenesis." These strategies have provided a wealth of information regarding the oncogenic potential of genes, and the sequence of events that leads normal cells down the path of malignant transformation. These investigations can be conveniently divided into two categories: insertion of viral oncogenes into mice, and insertion of endogenous protooncogenes and their transformed counterparts into the germ line. Both types of experiments have revealed oncogenic potential of genes as well as established systems for directing tumor formation to specific tissues.

Gordon, pages 199-204. Thus, the art clearly recognized the usefulness of transgenic animals, such as those of claims 40-45 and 59-62, for directing tumor formation to specific tissues and serving as animal models for cancer. *See also*, Aguzzi et al., *supra*.

Furthermore, the utility of the claimed transgenic mice is not limited to the development of an animal model for cancer. For example, the skilled artisan would recognize that applicants' transgenic animals, expressing an oncogenic, tumorigenic, or immortalizing protein, could be used to develop neuronal cell lines, as explained in the specification. (Specification, page 6, lines 12-15.) This is also consistent with the teaching of Camper et al., discussed above, which describe using cell-specific promoters to direct cell-specific expression of immortalizing oncogenes, such as the SV40 T-antigen, in transgenic mice. Camper et al., page 247, last paragraph. These mice are useful for developing immortalized cell lines that can be used, for example, to

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identify cell-specific transcription factors or to examine gene expression. *Id.* Similarly, Kioussis recognizes that this transgenic technology has been used to create tissue specific cell lines, thereby overcoming significant limitations in the previously existing technology, which relied on spontaneous or mutagen-induced tumor formation. Kiossis, p. 197.

As discussed above, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use. M.P.E.P. § 2164.01(c). The specification discloses at least two art-recognized utilities for the claimed transgenic mice—either as an animal model for testing anticancer drugs or for developing neuronal cell lines. In view of either of these utilities, the specification enables one of skill in the art to use applicants' claimed invention.

For the reasons discussed above, the specification provides an enabling disclosure that is commensurate in scope with the claimed subject matter. Accordingly, applicants respectfully request withdrawal of this 35 U.S.C. § 112, first paragraph, rejection.

Rejection Under 35 U.S.C. § 112, Second Paragraph

The Office also maintained the rejection of claims 43 and 44, under 35 U.S.C. § 112, second paragraph, for allegedly not pointing out and distinctly claiming the invention. (Paper No. 12, page 2.) Specifically, the Office contends that the rejected claims must recite "whether the DNA of the second mouse is endogenous or

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heterologous." (*Id.*) Although applicants respectfully disagree, in an effort to expedite prosecution, applicants have amended claims 43 and 44 obviating this rejection.

Double Patenting Rejection

The Examiner acknowledged applicants' request to hold the provisional double patenting rejetion in abeyance until Application No. 08/465,712 issues as a patent. (Paper No. 9, page 6.)

CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully request reconsideration and reexamination of this application and timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account no. 06-0916.

Respectfully submitted,

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